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Project Title: Toxin Effects on Genetic Diversity: TFM as a Model System

Project Category: Emerging Issues

Rank by Organization (if applicable): 0

Total Funding Requested ($): 218,555 **Project Duration:** 2 Years

Abstract:

We propose to determine the suitability of RAPD-PCR (randomly amplified polymorphic DNA-polymerase chain reaction) based measures of genetic diversity as an alternative to US EPA acute and chronic toxicity tests of ecological risk in a wider variety of resource types. This preliminary study will assess the amenability of a new set of test organisms, inhabiting streams in the Great Lakes ecosystems, to genetic diversity-based measures that sensitively, rapidly and inexpensively assay the impact that anthropogenic stressors have on aquatic species. We propose using sea lamprey control program as a model to test the feasibility using enhanced RAPD profiling techniques in ecological assesments of environmental quality. Specifically, we will determine the effect that chemical control of the sea lamprey with 3-trifluoromethyl 1-4-nitrophenol (TFM) has on populations of non-target invertebrate species. RAPD profiling will be performed on populations of non-target species inhabiting streams along each of the Great Lakes. Studying the population structure of species affected by the application of lampricides will be a true test of the utility of RAPD profiling as an indicator of population health. In order to demonstrate the utility of RAPD profiling, additional populations will also be tested from streams contaminated with a variety of other contaminants including PAHs and heavy metals. Genetic profiles will be visualized with traditional as well as innovative techniques. We will utilize ethidium bromide stained agarose gels and band fluorescence to produce RAPD profiles. By utilizing a portable fluorescence-detecting thermocycler, we will be able to generate profiles faster and less expensively than traditional methods would previously allow. Futhermore, the resulting profiles will be easier to analyze and more reproducible, allowing RAPDs to be used by a wider variety of agencies seeking to find more sensitive bioindicators of ecosystem health.

Geographic Areas Affected by the Project

States:

- [x] Illinois
- [x] Indiana
- [x] Michigan
- [x] Minnesota
- [x] New York
- [x] Pennsylvania
- [x] Wisconsin
- [x] Ohio

Lakes:

- [ ] Superior
- [ ] Huron
- [ ] Michigan
- [ ] Erie
- [ ] Ontario
- [x] All Lakes

Geographic Initiatives:

- [ ] Greater Chicago
- [ ] NE Ohio
- [ ] NW Indiana
- [ ] SE Michigan
- [ ] Lake St. Clair

Primary Affected Area of Concern: All AOCs

Other Affected Areas of Concern:

For Habitat Projects Only:

Primary Affected Biodiversity Investment Area: Not Applicable

Other Affected Biodiversity Investment Areas:

Problem Statement:

Currently accepted toxicity and biologically based indices have proven to be among the most sensitive and useful measures of environmental impacts through their direct assessment of the extent to which resource systems can harbor adaptable biological communities (McCarthy and Shugart, 1990). These indices on the biochemical, individual, population and community levels have demonstrated that both acute and chronic exposures to stressors can exert selective pressure upon organisms and that the bioavailability of pollutants is strongly correlated with decreased survival and reproductive success within populations (Burton, 1991). However, while toxicity measures, as well as species diversity and population densities, often return to normal levels shortly after remediation (Thorpe and Koonce, 1981; Allendorf and Leary, 1986), the population bottlenecks imposed by human activities can result in dramatic reductions in genetic diversity (i.e. Lavie and Nevo, 1982; Lavie et al., 1984; Nevo et al., 1986; Benton and Guttman, 1990) that are only restored through the comparatively slow processes of mutation and migration. Diminished genetic diversity is ultimately responsible for decreases in the resiliency of naturally occurring populations and their ability to efficiently utilize resources. The implications of diminished genetic diversity to a population can be profound. Free-living populations with high levels of genetic diversity utilize resources more broadly and efficiently (Beardmore et al., 1960; McDonald and Ayala, 1974). Genotypic variability within several species is also linked to such diverse features as the efficiency of oxygen transport, swimming performance and developmental rate (DiMichele and Powers, 1982; DiMichele et al., 1986; Hilbish and Koehn, 1985; DiMichele et al., 1991). Indeed, the ability of species to survive and adapt to changing environmental conditions is ultimately dependent upon its possession of a pool of genetic variability that can be drawn upon in response to selection from varying forces (Thorpe and Koonce, 1981; Allendorf and Leary, 1986; Parsons, 1989, Bradshaw and Hardwick, 1989, Baird et al., 1991).

Recent advances in molecular biology enabled the development of an assessment technique that has the potential of identifying both acute and chronic effects of pollution earlier than other presently used methods. This method uses RAPD-PCR (Random Amplified Polymorphic DNA-Polymerase Chain Reaction) to generate genetic profiles of individuals within populations. The combined analysis of the RAPD profiles can provide useful information regarding species endemic to sites impacted by pollution. RAPD profiles have proven effective in determining the overall genetic diversity levels harbored within populations of both aquatic and terrestrial species. By analyzing the genetic health of endemic populations, this protocol is capable of identifying populations at risk prior to their local disappearance. RAPD profiling is continuing to gain acceptance by both state and federal agencies including the Ohio EPA and US EPA.

Previous research has demonstrated the benefit of being able to apply a wide range of disciplines in addressing environmental concerns. The molecular-genetic approach that has been applied to ecological risk assessment has resulted in a method that has the potential to be more general and sensitive in assessing the extent that anthropogenic stressors have altered levels of overall genetic diversity in exposed populations. RAPD profiling is a good candidate method for the assessment of population health in that it has been found to generate very sensitive measures of genetic relatedness within populations of organisms. RAPD-PCR differs from conventional PCR in that it utilizes short primers to

amplify anonymous regions of DNA. The resulting DNA profiles can be: 1) readily analyzed due to differences in the sizes of the amplified regions; 2) reproducibly generated; and 3) used to distinguish between related individuals of the same species. The pattern of bands that result from PCR-amplification provides an excellent measure of a population's overall genetic diversity.

Proposed Work Outcome:

PROPOSED WORK: This study will further the test the feasibility of using RAPDs to determine the genetic health of populations of aquatic species that have been exposed to anthropogenic stressors. Specifically, the experimental design will use streams treated with lampricides as a model to test the ability of the RAPD bioassay to detect changes in the genetic population structure of non-target species affected by environmental contaminants. Members of the sea lamprey control program have expressed interest in the genetic effects of the treatment on non-target organisms (personal communication January, 2000). The lampricide control task force is administered by the Great Lakes Fisheries Commission (GLFC). The commission consists of officials from both Canada and several United States federal agencies including the US National Biological Service and the Department of the Interior. The US Fish and Wildlife Service (FWS) determines the frequency and the locations where TFM is applied based on juvenile lamprey population estimates. Typically, the application of TFM is rotated between streams in three to five year cycles.

The FWS continues striving to understand and reduce the non-target effects of their lampricidal agents. Terry Morse, the FWS program manager responsible for determining which streams will be annually treated with TFM, can provide all relevant information pertaining to this study. The treatment regime followed by the FWS provides an excellent opportunity to thoroughly examine the effects that lampricides have on the genetic health of populations of non-target species inhabiting treated ecosystems. This study will piggyback on the efforts of the lampricide control team. By working with the FWS, we will be provided the unique opportunity to monitor the genetic diversity profiles of test populations before, during and after the controlled introduction of an environmental contaminant.

The extent of the sea lamprey infestation and the corresponding control program make it possible to perform RAPD profiling on multiple species in each watershed during the period of this study. Where possible, we will collect crayfish and Hyalella populations (30 individuals per species) from each of the sites sampled in this study. We will sample several rivers and streams along each of the Great Lakes during the study period. During the first field season, we will focus on western Lake Erie, the western shore of Lake Huron, the eastern shore of Lake Michigan and the southern shore of Lake Superior along Michigan's upper penisula. We will work with the US FWS in the selection of both sample sites and additional sentinel test species. Three replicates of several study conditions will be utilized to follow changes in the genetic diversity levels of affected species. (1) Sample populations of crayfish, Hyalella and other test species will be collected from headwaters of streams scheduled for TFM treatment. Populations from these sites will be used for comparative purposes since TFM treatments are not performed in headwater areas. (2) Reference populations will be collected from sites directly upstream of the treatment sites. These sites will also be used to compare the effects that TFM had on the tested non-target species. (3) Sites immediately downstream of the treatment sites will be sampled for the test species. These populations should demonstrate a marked decrease in genetic diversity following the application of the lampricide. (4) Samples will be collected from locations significantly downstream of the treatment sites. These sites will be used to determine if the loss of genetic diversity is a localized event. (5) Populations of the sentinel species inhabiting 3 streams treated with TFM during the past two treatment periods will also be sampled. Where possible, sample populations will be collected in the headwaters, just upstream of the sample site and just downstream of the treatment locations.

RAPD assessment will be performed on the same sites the following summer to determine if genetic diversity levels of the indicator species are returning to pre-treatment levels. The second field season of the proposed project would involve more extensive sampling throughout all of the Great Lakes. Consistent with our previously mentioned study conditions, we will collect populations of two test species along three streams feeding Lake Ontario, eastern Lake Erie, the western shore of Lake Michigan and the northern shore of Lake Superior. Understanding that the same sentinel species may not be found in every ecoregion, we plan on working with various federal and state agencies to select the desired test species.

Additional streams will be selected for sampling in each Great Lake basin based on the extent they are affected by other environmental contaminants. We will select sample sites which our consistent with US EPA areas of concern (AOCs). Assessment of populations inhabiting rivers that are subject to TFM treatment, in addition to previously existing environmental stressors, will provide insight into whether there are cumulative effects on genetic diversity levels of non-target organisms. The effects of a wide variety of contaminants will be assessed. Specifically, populations of the indicator species will be collected from areas with known PCB, PAH and heavy metal contamination. Where possible, we will also test streams, treated by TFM, that are within the EPA's biodiversity investment areas (BIAs). Determining the genetic health of sensitive species inhabiting these coastal wetland ecosystems will provide resource managers with a

baseline for determining the health of these valuable ecosystems in the future.

EXPECTED RESULTS: RAPD-PCR profiles simultaneously survey a relatively large set of random genetic markers that are likely to be free from natural selection by stressors. Thus, they are more likely to provide a truer estimate of a population's genetic diversity than other molecular-based systems and be more sensitive than currently used ecological indicators. As a direct result, they should allow generalized detection of both chronic and acute effects of the lampricides and other environmental contaminants. RAPD profiles have previously been generated for populations sampled along pollution gradients from numerous Ohio rivers. Each of the sites that were previously studied in detail and the results of environmental analysis were readily available for comparison. The genetic profiles of the sample populations were compared to accepted measures of environmental impacts. Statistical analysis of the RAPD profiles indicates that genetic diversity is significantly correlated ($p<<0.001$) with accepted indicators of biotic integrity (Krane and Sternberg, 1999). Research is continuing on aquatic ecosystems throughout Ohio. The first practical application of the research was completed on the Black River in northern Ohio. This study was performed in cooperation with the Ohio EPA, US EPA, and USGS. In these studies, RAPD profiling proved to be a sensitive tool capable of assessing population health.

Although these initial studies demonstrated the potential for using the RAPD assay in environmental site assessments, they also revealed the need for automated analysis of the genetic profiles. Approximately 80% of the time required to perform the RAPD assay is spent during the final stages of the testing. Specifically, the gel electrophoresis and the manual scoring of the gels are both time intensive. The need for automation of the final analysis portion of the assay was apparent. Fortunately, the recent development of fluorescence-detecting PCR thermocyclers potentially eliminates the electrophoresis portion of the assay while automating the scoring of the genetic profiles. Automation of the genetic profiling will promote the future use of this bioindicator by making the method even more user friendly and reliable. Research and development of standard operating protocols (SOPs) for the environmental application of the Roche LightCycler is required to further refine the RAPD assay. This study will result in a method capable of assessing the health of aquatic populations faster and more sensitively than current techniques.

This project will also provide the framework necessary to perform long-term genetic diversity studies of species inhabiting each of the Great Lakes. The results generated during the two year period of this study will provide a baseline to measure future pollution induced changes in the genetic health of lake populations. Knowledge of a species current genetic diversity levels may have future impacts on both the individual tests species and the ecosystems as a whole. Since levels of genetic variability in populations enable populations to adapt to environmental stress, knowledge of diversity levels is useful in maintaining viable populations of aquatic species. More importantly, the sensitivity of genetic diversity levels will enable officials to use RAPD profiling as an assessment method capable of identifying environmental threats earlier than standardly used methods. As a direct result, the need for short-term field studies using measures of genetic diversity will become more prevalent. Finally, data collected would be invaluable to program managers charged with controlling exotic species while minimizing any effects on non-target endemic species.

The results of the study will be made available through an internet-based database that will provide a valuable reference tool to others performing ecological risk assessments. The web application will contain the genetic diversity levels for each species sampled throughout the Great Lakes. Information regarding the exact location of the sample collections (GPS coordinates) will be available through the web site. Additionally, we will publish the research results in peer-reviewed journals as well as Great Lakes conferences. All methodologies used to perform the genetic assessment will be made available in the form of SOPs. The database will also be available for government agencies (.gov extensions) to publish literature related to the Great Lakes and their watersheds. The web site and the knowledge gained through this project will be useful to other exotic species control programs as well as other officials charged with minimizing the effects of other anthropogenic stressors. The baseline genetic diversity levels will be useful for the foreseeable future. With both the baseline diversity levels and the SOPs, researchers will be able to periodically check the status of the indicator species and draw conclusions about the status of each Great Lake ecosystem.

Project Milestones:	Dates:
Project Start	11/2000
Site/species Selection - QAPP Approval	04/2001
Sample Collection/ Year 1	09/2001
PCR Optimization - SOP Development	01/2002
Sample Collection/ Year 2	07/2002
Completion of RAPD Profiling	09/2002
Web/Journal Publication of results	11/2002
Project End	11/2002

☐ Project Addresses Environmental Justice

If So, Description of How:

☒ Project Addresses Education/Outreach

If So, Description of How:

The proposed project will address the education outreach initiative valued by the US EPA on both a local and a national level. Funding provided by the GLNPO in conjunction with matching funds from Miami University will provide educational opportunities for both undergraduate and graduate level students. The students will be a valuable resource to the scientific community as the utilization of biotechnology in the environmental sciences continues to expand. Students involved with this project will further develop expertise using innovative molecular biology techniques to solve complex environmental problems. The skills that they master will increasingly be in demand by numerous federal and state agencies.

Nationally, researchers will benefit from the internet based knowledge presentation system that we will use to disseminate the results of the genetic profiling of the indicator species inhabiting streams in each of the Great Lakes. With the assistance of I.M. Systems Group, Inc., a premier software development firm, we will deploy a web page that will benefit every agency and academic institution interested in performing research on the Great Lakes in the future. The web-based system that we are proposing will not only contain the results of this project but will also be capable of disseminating literature from both past and future research studies. We will make it possible for the GLNPO to publish the final reports of all the research projects selected during this funding cycle. In fact, the proposed system will allow all federal agencies with reports regarding Great Lake ecosystems to publish their reports through our web-based database. The web site will be able to be linked to other related sites and will provide a central location for accessing information pertinent to the Great Lakes.

Project Budget:

	Federal Share Requested ($)	Applicant's Share ($)
Personnel:	77,340	46,615
Fringe:	5,848	6,489
Travel:	5,840	0
Equipment:	30,500	28,000
Supplies:	25,297	0
Contracts:	20,000	0
Construction:	0	0
Other:	0	0
Total Direct Costs:	164,825	81,104
Indirect Costs:	53,730	21,242
Total:	218,555	102,346
Projected Income:	0	0

Funding by Other Organizations (Names, Amounts, Description of Commitments):

The large scope of this proposed research effort is only possible due to the cost-sharing policies of Miami University and reutilization of software developed by the United States Air Force under a cooperative research and development agreement with I. M. Systems Group, Inc. This study will also utilize laboratory equipment that was originally purchased with funds supplied by a NCERQA Science To Achieve Results (STAR) Grant. Without the matching funds provided by the university and the application of a value-added approach to expenses previously incurred by US EPA and the Department of Defense, both the geographic scale of the study and our ability to disseminate the results would be diminished.

Miami University's Office of Research and Development has agreed to cost-share both equipment and personnel with the GLNPO. The university will provide nearly a third of the funding required to complete this project. Specifically, Miami's cost-sharing plan will amount to $102,346 of the $320,901 required to perform the genetic profiling of indicator species in each of the Great Lakes. The R&D office will provide more than half of the personnel costs in addition to $28,000 for equipment. More than 34K will be set aside by Miami for the training of graduate level students. Miami will fully fund the professional oversight of both the students and the project.

Description of Collaboration/Community Based Support:

We intend on collaborating with both the US EPA and US FWS sea lamprey program in the selection of sample sites and indicator species. This study will simultaneously test a sensitive bioindicator and identify effects of lampricides on non-target species. In order to disseminate the results of this and other projects funded by the GLNPO as widely as possible, we will collaborative with I.M. Systems Group, Inc. in the creation of a graphically oriented web-based database. The web site will contain the results of this study and where appropriate the final reports from other studies as well as other publications that are relevant to the protection of the Great Lakes. IMSG is a small minority-owned business with a proven track record of providing high-quality Information Technology professional services. Although a small company, IMSG has an outstanding record of consistent project successes and client satisfaction. They have recently been certified under both the ISO 9000 quality, and the international TickIT software quality standards. The quality of their efforts is reflected by:

- IMSG's staff receiving Vice President Gore's "Hammer Award" for team excellence and innovation in 1999.
- The Air Force's nomination of them in 1995 as "Small Business Prime Contractor of the Year"
- The Small Business Administration award to them of the "Administrator's Award for excellence" in 1996.

They provide significant and recognized capability to create leading edge technical innovations in the web-based application of knowledge presentation systems. They offer, among other capabilities, to reuse and extend related applications originally created under a Cooperative Research and Development effort with the United States Air Force. The reutilization

of previously developed software dramatically reduces the costs associated with the creation of the proposed web application.